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## REMARKS

Examiner has rejected Claims 1-5, 7, and 8, now pending in the application, under 35 U.S.C. 102(b) as being clearly anticipated by Murshudov et al. Applicant respectfully traverses these rejections. However, Applicant has revised Claims 1,2,3, and 7 to clarify and distinguish Applicant's invention from noted prior art references as discussed with Examiner during the telephonic conference held on July 20, 2005.

The parties involved in the telephonic conference were Applicant - Thomas Terwilliger, Applicant's representative - Mark N. Fitzgerald, Examiner - Eric S. DeJong, and Examiner John Brusca. All of the currently pending claims [Claims 1-5, 7, and 8] were discussed during the telephonic conference.

After introductions, Examiner DeJong requested Applicant summarize the main points of the proposed response to the June 30, 2005. Applicant first summarized the Examiner's general argument for rejection of the application, which was that Murshudov et al. describes a process known as "macromolecular refinement" that leads to improvement in electron density maps, and which has some similarities to the methods in the application.

Applicant described how the method in the pending application is different in fundamental ways from the method taught in Murshudov et al. Applicant discussed how the improvement of electron density maps using refinement and using density modification use different sets of parameters to describe the crystal structure and because of this they have different properties.

Applicant further described how in macromolecular refinement the parameters are coefficients in a model describing the electron density map. Refinement consists of adjusting the parameters to improve the agreement between structure factors calculated from the model and those measured in an experiment. In density modification the parameters are the crystallographic phases. In density modification the crystallographic phases are adjusted to improve the agreement between an electron density map calculated using these phases and expectations about the features of this

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electron density map, such as a flat solvent region. The adjustments in refinement therefore are directed towards matching calculated amplitudes to experimental amplitudes, while those in density modification towards matching calculated electron density to expected electron density. This is the fundamental difference between the approach of Applicant and Murshudov et al. Applicant and Examiner Dejong discussed these points to clarify them, and there appeared to be general agreement that Murshudov et al. does not anticipate the main aspects of Applicants invention.

Examiner Dejong and Examiner Brusca suggested several changes to the wording of the proposed amendment to the Claims to make them more clear, to include removing the words "containing errors" in Claim 1b and replacing them with "of the crystal structure", and removing "first" in claim 1c to properly reflect the iterative aspects of the method.

Lastly, Examiner Dejong suggested that addition prior art might be relevant to the application, and cited the paper Roberts, A.L.U. and Burner, A.T. (1995) Acta Cryst D51, 990-1002 as an example.

As a result of the telephonic interview with Examiners Dejong and Brusca, the following response to the Office Action dated June 30, 2005 is presented:

Claim 1:

Applicant notes Examiner's general argument that Murshudov et al. teaches an iterative process known as macromolecular refinement that improves an electron density map, however, Applicant stresses that there is a significant difference between the "refinement" of a macromolecule as taught in Murshudov et al. and "density modification" to remove atomic model bias as it is taught and claimed by applicant.

Applicant notes three features of electron density maps and models:

(1) A crystallographic structure can be completely described by either an electron density map or by a set of crystallographic phases and amplitudes. These can be interconverted using a Fourier transform.

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(2) A model can be used to describe an electron density map. Based on the coefficients in the model, an electron density map can always be calculated from a model of a structure.

(3) The experimental data normally consists of crystallographic amplitudes alone. The improvement of electron density maps using refinement and using density modification use different sets of parameters to describe the crystal structure and because of this they have different properties.

In macromolecular refinement as taught in Murshudov et al., the parameters are coefficients in a model describing the electron density map. Refinement consists of adjusting the parameters in such a model to improve the agreement between structure factors calculated from the model and those measured in an experiment.

In contrast, in density modification as taught and claimed by Applicant the parameters are the crystallographic phases. In density modification the crystallographic phases are adjusted to improve the agreement between an electron density map calculated using these phases and expectations about the features of this electron density map, such as a flat solvent region.

The adjustments in refinement therefore are directed towards matching calculated amplitudes to experimental amplitudes, while those in density modification towards matching calculated electron density to expected electron density. This is the fundamental difference between the approaches.

The methods are different in a specific way as well. A key aspect of density modification as taught and claimed by Applicant is that crystallographic phases are derived in elements d-g of Claim 1 from features such as the flatness of the solvent, and are not derived from an atomic model for the macromolecule. In contrast, in the method of Murshudov et al the phases ( $\phi_c$ ) used to calculate an electron density map (Eq. 20 of Murshudov et al) are calculated from directly from the model after refinement. This difference is central to the purpose of the density modification as taught and claimed by Applicant, which is to obtain an electron density map that has little bias from a model.

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Applicant has revised Claim 1 to further define the present invention's ability to perform density modification, especially to include flatness of a solvent region (for which support is provided in Page 9 of the specification), as opposed to macromolecular refinement.

The examiner states that "Murshudov et al. further provides examples of using the above calculation in building partial models built on experimentally phased maps and using said maps to generate a useful contribution to the calculated structure factor (step (c) deriving an electron density map from the first set of structure factors ; step (d) identifying features of the electron density map to obtain expected distributions of electron density ; step (e) making a comparison between the electron density map and the expected distributions of electron density) . See Murshudov et al., page 243, column 2, lines 1-23, page 248, figure 2, and page 249, column 1, lines 1-15"

Applicants note that in this pending application the meaning of (d) identification of features of the electron density map and (e) making a comparison between features and the map is not simply to notice the presence of atoms or the presence of a helix or the presence of solvent. It is to specify an idealized abstraction of those features and their location in mathematical form as in Equations 3 and 4 and to use these mathematical relations to assess to probability that the map is correct. The specification on page 10 emphasizes the importance of these steps: "The probability distributions for electron density given that a point is in the protein or solvent regions are central to map-based phasing. They define the expectations about electron density in the map." This concept of a probability that the density at a particular point in the electron density map is correct is *not* taught by Murshudov et al.

Further, regarding the specific citations presented by the examiner: Murshudov et al ., page 243, column 2, lines 1-23 describes the use of partial models to estimate structure factors for the complete correct model. It does address (c) deriving an electron density map. This section does *not* address (d) identification of features of the electron density map as described above or (e) making a comparison between features and the

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map.

Murshudov et al. page 248, figure 2 shows sections of electron density maps calculated using 2Fo-Fc, SIGMAA, and REFMAC map calculation procedures. These sections show the results of carrying out the refinement procedures described by Murshudov et al. on the quality of the final electron density map. The figure does not demonstrate (d) identification of features of the electron density map as described above or (e) making a comparison between features and the map.

The examiner states that, "In examples applications of the disclosed methodology, Murshudov et al. teach the evaluation of differences between calculated phases determined from current and final models at all stages of the refinement process, which reads on the claimed step (f) estimating how changes in the crystallographic phase of a reflection  $k$  affect the comparison. See Murshudov et al., page 246, column 1, line 15 through column 2, line 2."

Applicant believes that Murshudov et al. page 246, column 1, line 15 through column 2, line 2 is a discussion of how the authors evaluate the quality of the refinement process that they have described. They use the difference between phases calculated from a model at intermediate stages in refinement and those at the end of the refinement to assess progress. Further they calculate electron density maps using coefficients  $mFo-DFc$  and  $2mFo-DFc$  which they compare with the  $Fc$  maps obtained from final coordinates.

In the analysis of Murshudov page 246, column 1, line 15 through column 2, line 2, the "final" electron density map is known in advance, as it has been obtained from a previous analysis of the structural data, as described in Murshudov et al. Table 2 caption: "mapc, map correlation between named map, and the  $Fc$  map from the deposited coordinates." In a real structure determination the "final" map is not known until the determination is over. The comparison made by Murshudov et al. is unlike the comparison made in the present application in elements d and e of claim 1 in which features are identified in the current electron density map without using any "final"

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electron density map as a reference.

The purpose of the comparison of Murshudov et al. page 246, column 1, line 15 through column 2, line 2 is simply an overall evaluation of whether their method is working or not. There is no analysis (d) identification of features of the electron density map as described above or (e) making a comparison between features and the map or (f) estimating how changes in the crystallographic phase of a reflection  $k$  affect the comparison.

The Examiner states that "Murshudov et al., discloses derivative forms of the log-likelihood which rely on an evaluation of the prior distribution of phases,  $p(4)$ , and is utilized in evaluating the minimization of the maximum likelihood function that is utilized in the above described refinement procedures (element g establishing crystallographic phase probability distributions from the comparisons for the possible crystallographic phases of reflection  $k$ ). See Murshudov et al., page 251, column 2, line 1 through page 252, column 2, line 26"

Murshudov et al., page 251, column 2, line 1 through page 252, column 2, line 26 describe derivatives of a log-likelihood function. There are an infinite number of possible log-likelihood functions. The log-likelihood function described by Murshudov is the one shown in their Equations 13 and 14, page 244. This log-likelihood function is based on the values of amplitudes calculated from a model and observed amplitudes. There is no electron density map, and there is no analysis of features of an electron density map. In contrast the log-likelihood function described in applicant's application is given by Equations 3 and 4 in the specification. This log-likelihood function is based on the electron density map and corresponding features. Thus, the two types of log-likelihood functions are completely different, and, therefore, the derivatives calculated by Murshudov et al. do not have any bearing on applicant's present application.

The examiner states that, "Contrary to applicants assertion that Murshudov et al. does not teach that certain parts of the electron density map are known accurately and

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other parts are not, as stated above, Murshudov et al, teaches that in the structure refinement and modeling procedure, accommodating identified regions of structure and bulk solvent is handled by additions to the structure factor by modifying equation 9, the expression for probability distribution. See Murshudov et al ., page 250, column 2, lines 1-41 ."

The examiner is correct in that Murshudov anticipates that different components of the model can have different errors. Although Murshudov does not specifically relate this to different locations in the map, the extension is straightforward.

The examiner states that, "Further, the instant claims do not contain any limitation restricting the identification features in the electron density map to exclude the presence of the atoms in the current atomic model of the macromolecule. Therefore, Applicants assertion that the features that are identified in the electron density map are normally not the presence of the atoms in the current atomic model of the macromolecule, but rather a different set of features that yield independent information, and it is the use of this independent information is not taught by Murshudov et al . is not found convincing ."

Applicant notes that the claims, taken together with the specification, do make it clear that the features that are identified in the electron density map are normally not the presence of the atoms in the current atomic model, but rather a different set of features that yield independent information:

Claim elements 1a and 1b state that a first set of structure factors and electron density come from "selecting a starting set of crystallographic phases to combine with the observed structure factor amplitudes ". The specifications emphasize that the starting crystallographic phases typically come from the starting atomic model by stating that: Page 5 "A starting set of crystallographic phases is selected from a model or other source ". Further (Page 4) the specifications explain the significance of starting with phases from a model: "Bias of this kind

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commonly occurs when crystallographic phases are calculated based on a model that contains atoms that are incorrectly placed "

While the starting phases typically come from a model, the features are normally not those of the model. Claim element 1d is "identifying features of the electron density map to obtain expected distributions of electron density", and the specifications clarify this by giving the simplest case in which the solvent flatness is the only criteria and no reference to atoms is made at all: (Page 9)

"For example, a simple map likelihood function might be based on defined regions of an electron density map containing the macromolecule (with substantial variations from point to point) and containing solvent (with a very uniform electron density). A value of the phase for a particular reflection  $k$  that leads to a map with a relatively flat solvent is more likely to be correct than a phase that does not."

The specifications further expand upon this separation of the information used to obtain the first map (typically atomic coordinates with model bias) and the information used to obtain phase information (typically solvent flatness or density probability distributions, not based on atomic coordinates). The basis for this rationale is explained in the detailed description:

Page 6: "In principle, however, experimentally derived or other prior phase information does not necessarily need be included in the maximum-likelihood density modification procedure. Instead, phase information can be derived from the agreement of the map with general expectations alone."

and the separation of prior phase information (from the model) and new information (from the map) is emphasized:

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Page 8: "The effect of each cycle in this procedure is to obtain a probability distribution for each phase independently of all the others, based on the agreement of the electron density map with expectations. In the phase probability calculations, all possible values of the phases are considered without any preference for the values used in the previous cycle. "

Applicant notes the reference of Roberts, A. L and Brunger, A. T. (Acta Crystallographica (1995), D51, 990-1002 that teaches the use of density modification to improve the quality of electron density maps, and the use of the free R value to limit the potential of overfitting, *does not* teach or suggest the elements d, e, f, or g of Claim 1.

Roberts and Brunger do not teach elements d, e, or f of Claim 1 because they do not construct probability functions for electron density for each point in the electron density map. The method of density modification taught by Roberts and Brunger (reference section 3.1 "Solvent-flattening procedure") consists of replacement of electron density in the solvent region with its average value and by truncation of negative electron densities, followed by inverse Fourier transformation to obtain a new set of phases. In their procedure there is no construction of probability functions for electron density; no comparison between the electron density map and the expected distribution of electron density probability functions; and no estimation of how changes in the crystallographic phase of a reflection  $k$  affect this comparison.

Further, Roberts and Brunger do not teach element g of Claim 1. The key element of Claim 1g is that crystallographic phase probability distributions are obtained from the comparisons made in elements e and f of Claim 1. In this calculation the phase information from the starting model is not used. Roberts and Brunger do not teach this element because the inclusion of phase information from the starting model is intrinsic to their method. Roberts and Brunger describe the method of solvent flattening in section 3.1 "Solvent-flattening procedure", which states in part that, "Phases obtained by inverse Fourier transformation of this flattened and truncated map

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are then recombined with the initial phase information to produce a less biased phase estimate." This recombination step uses the phase information from the starting model. This is exactly the information that is not used in Applicant's claimed method because this information biases the map towards the starting model. The degree to which inclusion of phase information from the starting method is central to the method of Roberts and Brunger is emphasized by their Equation (6), in which the combined phase probability is equal to the initial phase probability multiplied by the phase probability distribution of solvent-flattened phases, and by their Figure 1, in which "combine phase probabilities" is a central step.

Applicant notes further that Claim 8 comprises a method to use a small amount of the biased information from the starting model in element g of Claim 1. This method is different from the method of Roberts and Brunger that uses the full initial phase information in their corresponding step. This is shown in their Equation (6), in which the combined phase probability is equal to the initial phase probability multiplied by the phase probability distribution of solvent-flattened phases.

However, In order to make element g of Claim 1 as clear as possible, Applicant has added the phrase, "without including phase information from the starting model of the crystal structure;" to emphasize a difference between Applicant's claimed method and other density modification methods. Applicant notes that this difference is made clear in the specification, which states that, "this phase probability calculation estimates how the log-likelihood of the map will vary with the phase of reflection  $k$  without regard to the value of the phase that was used to calculate the original electron density map", and, ". In the phase probability calculations, all possible values of the phases are considered without any preference for the values used in the previous cycle."

Claims 2, 3, 4 and 5:

Each of these claims describes a class of features of an electron density map that can be used in Claim 1, elements d, e, f, and g to obtain and use a probability distribution for electron density. Murshudov et al. does teach the existence of each of

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these features of a map, however, Murshudov et al. does not teach their use in obtaining a probability distribution for electron density. Further, as Claim 1 is now a novel allowable claim these dependent claims are now allowable as well.

Claim 7:

Murshudov et al. teaches the use of maximum-likelihood functions and FFT methods for calculation of derivatives for refinement of macromolecular structures, but does not teach their use in estimating phase probabilities from probability distributions for electron density. Further, as Claim 1 is now a novel allowable claim this dependent claim is now allowable as well.

Claim 8:

Murshudov et al. teaches the use of a model structure having similarities to the crystal structure being examined and the use of weighting factors to reduce model bias, but does not teach the use of either in the method of Claim 1. Stated another way, as Claim 1 is now a novel allowable claim this dependent claim is now allowable as well.

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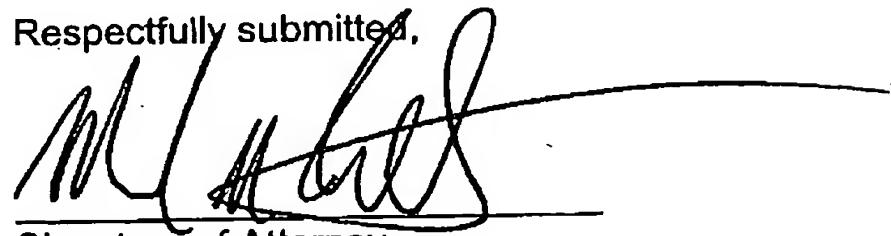
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Therefore, the Examiner is requested to allow Claims 1-5, and 7-8, and to pass this case to issue.

Applicant's attorney would be pleased to further discuss this matter by telephone with the Examiner if the Examiner concludes such a discussion would assist in moving this case to issue.

No new matter has been added as a result of this response.

Respectfully submitted,



Signature of Attorney

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Reg. No. 48,300  
Phone (505) 665-3112

Mark N. Fitzgerald  
Los Alamos National Laboratory  
LC/IP, MS A187  
Los Alamos, New Mexico 87545